THE IMMUNE STATUS OF TWO BLOOM'S SYNDROME SIBLINGS

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by

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An abstract of a Thesis by
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The problem. Two sibling brothers have been previously diagnosed as Bloom's Syndrome (BS). Due to lack of demonstrable increased sister chromatid exchange (SCE) in their blood cells and fibroblasts, diagnosis of BS is in question.

Procedure. This study examined the immune reactions of these siblings with such tests of immune function as: T- and B-cells, T-cell subsets, chemotaxis, nitroblue tetrazolium (NBT), mitogen stimulation and mixed lymphocyte culture (MLC).

Findings. Results are compared with those of other known BS subjects. Analysis of our patients' immune systems seems to agree with findings of previous studies.

Conclusion. While there is a correlation of immunological and clinical data, genetically there are none of the expected abnormalities. This study has not ruled out the possibility of a misdiagnosis of BS.

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INTRODUCTION

Dr. David Bloom first noted a Bloom's Syndrome (BS) patient in 1941. The patient was described as a dwarf who presented an eruption on the face resembling lupus erythematosis in addition to a bullous eruption on the lips due to hypersensitivity to sunlight (Bloom 1954). In 1953 two more cases were presented to the New York Academy of Medicine. It was believed at the time that three such identical cases might constitute a syndrome.

Dr. Bloom's original male patient, aged nine years, was born to Jewish parents and had a facial skin eruption from the age of two years. Born full term at four pounds the patient weighed only nine pounds at one year. At nine years he was the size of a small four year old with a narrow face giving the appearance of precocious senility. The areas of the face not affected by erythematous rash appear wrinkled, the rash assuming a butterfly shape. On the patient's trunk were numerous cafe au lait spots. The patient was treated with growth hormone from 1941-1943. In 1948 he had grown eight and one-half inches and was a second-year high school student.

German and Bloom (1977) were the first to associate the risk of cancer with Bloom's. A BS Registry had begun in 1963 when the second of the three first cases died of acute leukemia. By 1976 there were 32 recognized as Bloom's Syndrome and at the end of 1979 a total of 71 were

registered. Only those persons presenting the cardinal features defined by Bloom in 1966 were accepted into the registry. These symptoms included congenital telangiectatic erythema of the face, sensitivity to sunlight and stunted growth (Bloom 1966).

As early as 1963 it became clear that cellular chromatid interchange was a characteristic finding in BS. About 1974 it was discovered that the number of sister chromatid exchanges (SCE) in dividing BS lymphocytes were many times greater than in normal lymphocytes. The SCE phenomenon is now known to be a cancer-related condition (Siniscalco 1977).

In the patients followed by Bloom and German, leukemia was the type of cancer found in nearly half of the cases. Leukemias appeared at earlier ages than did solid tumors. Bloom and German felt that BS individuals may have the greatest predisposition to cancer of all the known recessively transmitted disorders of man.

Today we know that BS is a rare autosomal recessive disorder in which the affected individual is at increased risk of cancer. The major features of this syndrome are: growth deficiency (including prenatal growth retardation with low birth weight), short stature, characteristic sun-sensitive telangiectatic erythema of the face and other exposed areas of skin. Cultured BS fibroblasts have retarded DNA-replication fork displacement and there is a

greatly increased frequency of SCE and an elevated spontaneous mutation rate (Lechner et al. 1983).

The purpose of this project was to examine the immune response in two male siblings (ages seven and six years) diagnosed as having BS. Little literature has been published dealing with the immune function of such patients. Previous work suggests that both humoral and cellular immune responses are affected. There is a pattern of abnormal serum concentration of at least one class of immunoglobulins, an impaired response to pokeweed mitogen stimulation and a mixed lymphocyte culture (MLC) showing BS cells to be normal stimulators but poor responders. These defects in the immune system may result in multiple infections for the BS patient. These two patients have displayed frequent bouts of otitis media, upper respiratory infections and gastro-intestinal complications.

METHODS AND PROCEDURES

Blood in heparinized and ethylenediaminetetracetic acid (EDTA) tubes was drawn from the two BS patients and their parents along with a normal control. The blood for chemotaxis and nitroblue tetrazolium (NBT) tests was separated. Remaining blood was prepared on a density gradient to isolate lymphocytes for use in T- and B-cell enumeration, T-cell subsets, mitogen stimulation and mixed lymphocyte culture. Heparinized blood was layered in 10 ml amounts on density gradient and spun in the centrifuge at

400 xg for 30 minutes. The layer of mononuclear lymphocytes was then harvested for testing.

Chemotaxis

One or two ml of blood specimen in EDTA from patients, parents and control were incubated in appropriate vials of N-Formyl-L-methionyl-L-phenylalanine (F-met-phe) purchased from Sigma Chemical. Final dilutions were 50 and 100 picamoles of F-met-phe.

The samples were mixed and incubated in the vials at room temperature for 30 minutes. They were then remixed and monolayer films of blood prepared on glass slides. Slides were stained with Wright's stain and examined under a light microscope for the presence of bipolar-shaped neutrophils. One hundred neutrophils were counted on each slide and the percent of bipolar cells was recorded (Jadwin et al. 1981).

NBT (Nitroblue tetrozolium test)

Heparinized blood (1.5 ml) was placed in two test tubes. To one of the tubes 15 µl of endotoxin was added as a stimulant. All tubes were then incubated at 37°C for 10 minutes in a waterbath. NBT solution (0.3 ml at 0.15%) was added and blood was run through nylon wool columns, rinsed twice with phosphate buffered saline then once with distilled water to lyse the red blood cells. A few drops of 0.1 M HCl was placed in each column to stop the reaction. Then columns were rinsed again with 2 ml of distilled water. Extracted nylon wool was placed in test tubes

containing 5 ml of dioxane. Dioxane tubes were heated in a 70°C water bath for 20 minutes with frequent shaking to extract the blue formazan product. Tubes were centrifuged to remove any nylon wool fibers. The optical densities of the solutions were then measured at 520 nm on a spectrophotometer.

T- and B-cells

For T- and B-cell assays, mononuclear cells were incubated with latex particles to mark the monocytes.

T-cells were tested for by the E-rosette procedure. Cells (5×10^5) were mixed with 0.1 ml of sheep red blood cells (SRBC) and incubated for 10 minutes at 37°C. SRBC were at a concentration of 25 µl of packed cells/5 ml diluent. The mixture was then centrifuged at 200 xg and incubated overnight at 4°C. The next morning 10 µl of trypan blue stain was added and the cells were mixed gently by Pasteur pipette and loaded into a hemocytometer for counting. Rosettes consisting of a lymphocyte with 3 or more attached SRBC were enumerated and percentages recorded.

For B-cells, 200 µl of mononuclear cells at a concentration of 1×10^7/ml were mixed with FITC conjugated goat anti-human IgG F(ab)'2 (Cappel Laboratories), incubated at 4°C for 30-40 minutes then washed with a 20% solution of sodium azide three times. A drop of cell suspension was placed on a glass slide, covered with a coverslip and edges sealed with nail polish. B-cells were counted using the

fluorescent microscope and the percent of Ig positive cells were noted.

T-cell Subsets

T-cell subsets were detected using monoclonal antibodies. Cells (1×10^7) were mixed with 10 µl of reagent for the T-helper/suppressor ratio test (Becton/Dickinson) and incubated for 30 minutes at $4^{o}C$ in ice. T4 (helper) and T8 (suppressor) cells were counted simultaneously, the T4 fluorescing as orange and the T8 as green. A total of 100 cells were counted and the results were recorded both in percent and ratio form.

Mitogen Studies

Mononuclear cells were separated from heparinized whole blood using sterile technique. All tubes, solutions and pipette tips were sterile. The cells were diluted in stimulation medium consisting of: 0.5 ml pen/strep (penicillin 5000 units, streptomycin 5000 µg/ml), 0.5 ml L-glutamine (200 mM) in 50 ml of RPMI 1640 culture medium. The final dilution was 2×10^5 cells/ml.

Mitogen dilutions for phytohemagglutin antigen (PHA) were: 0.125, 0.5, 1.0, 2.0, and 4.0 µg/ml. Conclavalin A dilutions were: 2.5, 5.0 10.0, 20.0, and 40.0 µg/ml.

Microtiter plates were prepared as follows:

20 µl control serum (pooled human AB)

100 µl leukocyte suspension

80 µl mitogen dilutions

All tests were done in triplicate. The solutions were mixed by a gentle tapping of the plates which were then incubated at 37°C in 5% CO_2 for 3 days. Radioactive thymidine (New England Nuclear) in 10 µl amounts (at a concentration of 1 uCi/10 µl) was added to each well and the plates were incubated a further 4 hours. Cells were harvested by Mash Cell Harvester on fiberglass filters. Filter punches were placed in scintillation vials, 3 ml of scintillation fluid added and radioactivity was counted on a Beckman beta counter. Stimulation indexes were calculated and reported in graph form. Stimulation indexes were determined by the formula:

$$SI = \frac{\text{Mean counts/min for mitogen}}{\text{Mean counts/min for control}}$$

Pokeweed mitogen (provided by Drake University) was diluted as per instructions and used in 3 dilutions of 1/10, 1/100, and 1/1000. The same techniques were employed as for the previous mitogen tests except the incubation period was 5 days.

Mixed Lymphocyte Culture (MLC)

Separated lymphocytes were divided into two portions for patients, parents and control. One portion of cells was treated with mitomycin C (Sigma Chemical) at 0.25 mg/ml and incubated for 20 minutes at 37°C in 5% CO_2. The cells were then washed 3 times in culture medium and designated "stimulator cells." The final concentration of both cell

suspensions was 5 x 10^5 cells/ml in culture medium with 20% pooled human serum (PHS).

The test was set up in microtiter plates with all possible combinations of cells done in triplicate. Stimulator cells were placed in the plates' vertical rows and responder cells were added to horizontal rows. At the end of each row vertically and horizontally were blanks (designated PHS) consisting only of cells and medium.

Set up of microtiter plates:

(Responder cells)	(Stimulator cells)					
	Father	Mother	Sibl	Sib2	Control	Blank
Father	----					
Mother		----				
Sibl			----			
Sib2				----		
Control					----	
Blank						----

The plates were mixed gently by tapping and incubated for six days at 37°C in 5% CO_2. At the end of the incubation period thymidine (at the same concentration as in the mitogen stimulation) was added to all wells and plates were incubated an additional six hours. Plates were harvested as in the mitogen procedure above and counted in a

beta counter.

RESULTS

Results of the chemotaxis studies are shown in Table 1 as the percent of neutrophils exhibiting bipolar configuration. Counted as bipolar were those cells with distinctly elongated shapes or with the presence of a uropod, indicating reactivity to the f-met peptide. None of the results varied greatly from that of the normal control though sibling 1 (Sib 1) seemed to have the least reaction to chemotactic factor as compared to the others.

The NBT results are noted in Table 2. A stimulation index for stimulated cells should be approximately 2-4 times that of non-stimulated cells. The father showed the lowest stimulation index at 1.1. With stimulation indexes of 2.5, 2.8, and 3.0, respectively, the mother and siblings were well within normal ranges so we find basically normal phagocytic capabilities.

T- and B-cells were mostly within normal ranges. As illustrated in Table 3 the absolute values for T- and B-cells for our subjects fall basically within the limits set by our laboratory according to a previous study based upon normal patients of various age groups. The only number out of the expected normals was that for the B-cell count of Sib 1. The absolute value of 44 was considerably below the normal range of 125-684.

Table 1. Results of chemotaxis test reported in percent of bipolar neutrophils observes.

F-met-phe	Father	Mother	Sib 1	Sib 2	Control
50 pM	35%	30%	22%	30%	34%
100 pM	44%	39%	28%	52%	49%

Table 2. The findings of the NBT assay recorded both as O. D. reading and stimulation index (calculated by dividing O. D. reading of stimulated sample by O. D. reading of non-stimulated sample).

O. D.	Father	Mother	Sib 1	Sib 2	Control
Stim	0.075	0.125	0.070	0.030	0.025
Non-Stim	0.070	0.050	0.025	0.010	0.010
Stim Ix	1.1	2.5	2.8	3.0	2.5

Table 3. T- and B-cell counts expressed as percentages and absolute value. Absolute values are the lymphocyte count x the percent of cells enumerated. Normals are based on tests performed on normal patients of the same age group.

	% E-rosettes	Absolute Value	Normals for age	% B-cells	Absolute Value	Normals for age
Father	59%	1926	385-1723	5%	163	48-330
Mother	80%	2464	385-1723	5%	154	48-330
Sib 1	60.5%	1316	875-2628	2%	44	125-684
Sib 2	46.5%	1281	875-2628	6%	165	125-684
Control	68%	885	385-1723	5%	65	48-330

Table 4. T-cell subsets as expressed in percent and T4/T8 ratio.

Subset	Father	Mother	Sib 1	Sib 2	Control
T4/helper	77%	71%	81%	60%	78%
T8/suppressor	23%	29%	19%	40%	22%
Ratio	3.35	2.24	4.26	1.50	3.55

T-cell subsets seem to be normal also as noted in Table 4. The average T4/T8 ratio is 1.9 ± 0.7. All the ratios for this test fall within these limits though Sib 2 is in the lower ranges of normal.

The mitogen stimulation presents varied results. As seen in Figures 1 and 2, both parents and control have a good reaction to mitogens PHA and Con-A, though the father's response was not as marked as the mother's. Sibling 1 responded only fairly to PHA and Con-A, while Sibling 2 had a moderate response to both mitogens. Figure 1 illustrates responses to PHA as expressed by the stimulation index. Figure 2 notes the stimulation indexes at the various concentrations of Con-A.

The sibling B-cell response to Pokeweed mitogen was poor. One may note in looking at Figure 3 that the parents had excellent responses to the mitogen, even greater than that of the control, while those of the siblings were nearly flat at all three concentrations.

In Table 5 counts per minute (cpm) for the mixed lymphocyte culture (MLC) are recorded. While several of the counts were obviously in error (possibly due to contamination or pipetting error), one can see a definite trend on the part of the two siblings to be poor responders. By looking at the stimulation indexes indicated in Table 6 one notes in the horizontal rows (responder cells) obvious lack of response by the cells of the BS

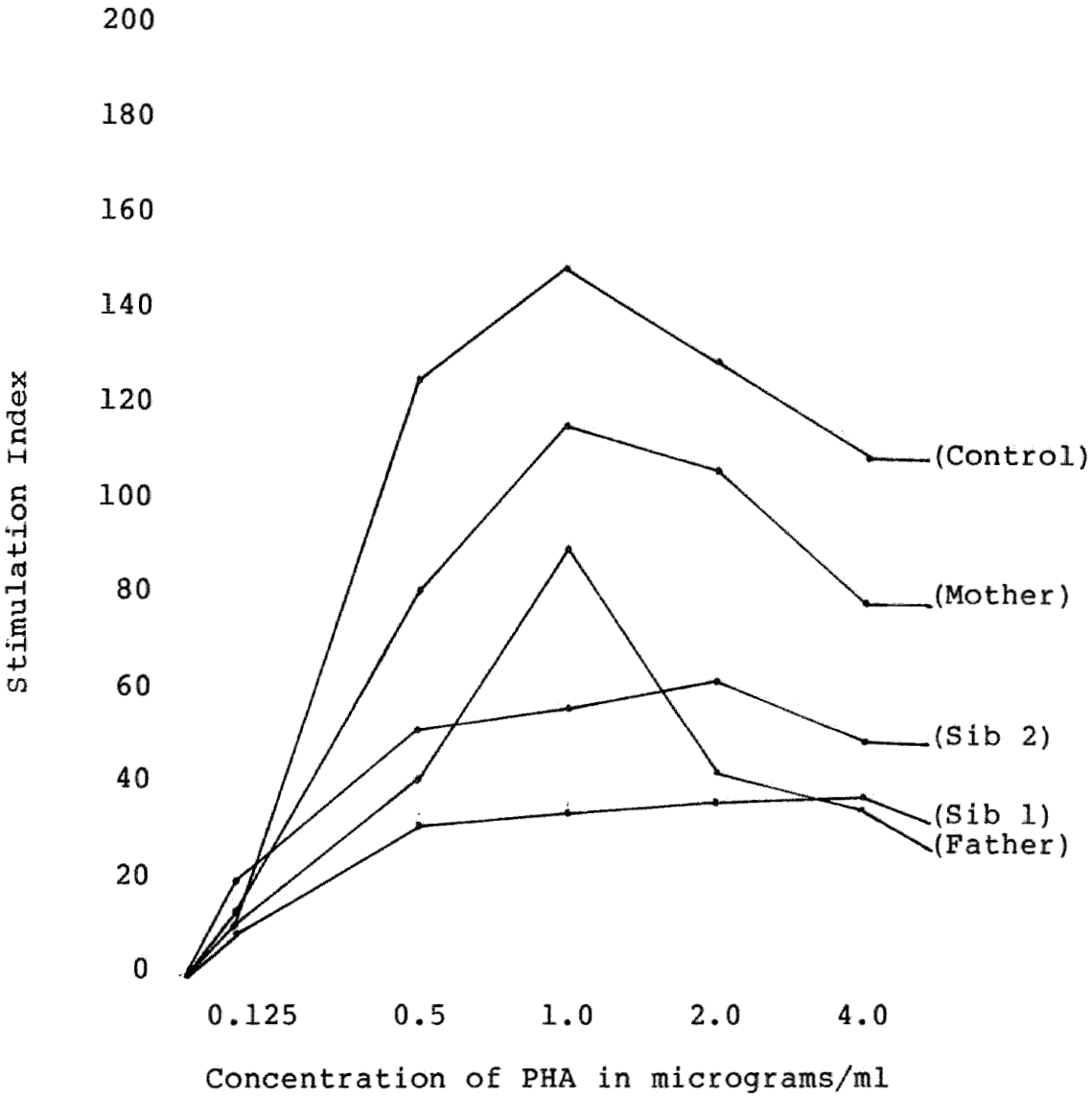

Figure 1. Stimulation indexes for the mitogen PHA.

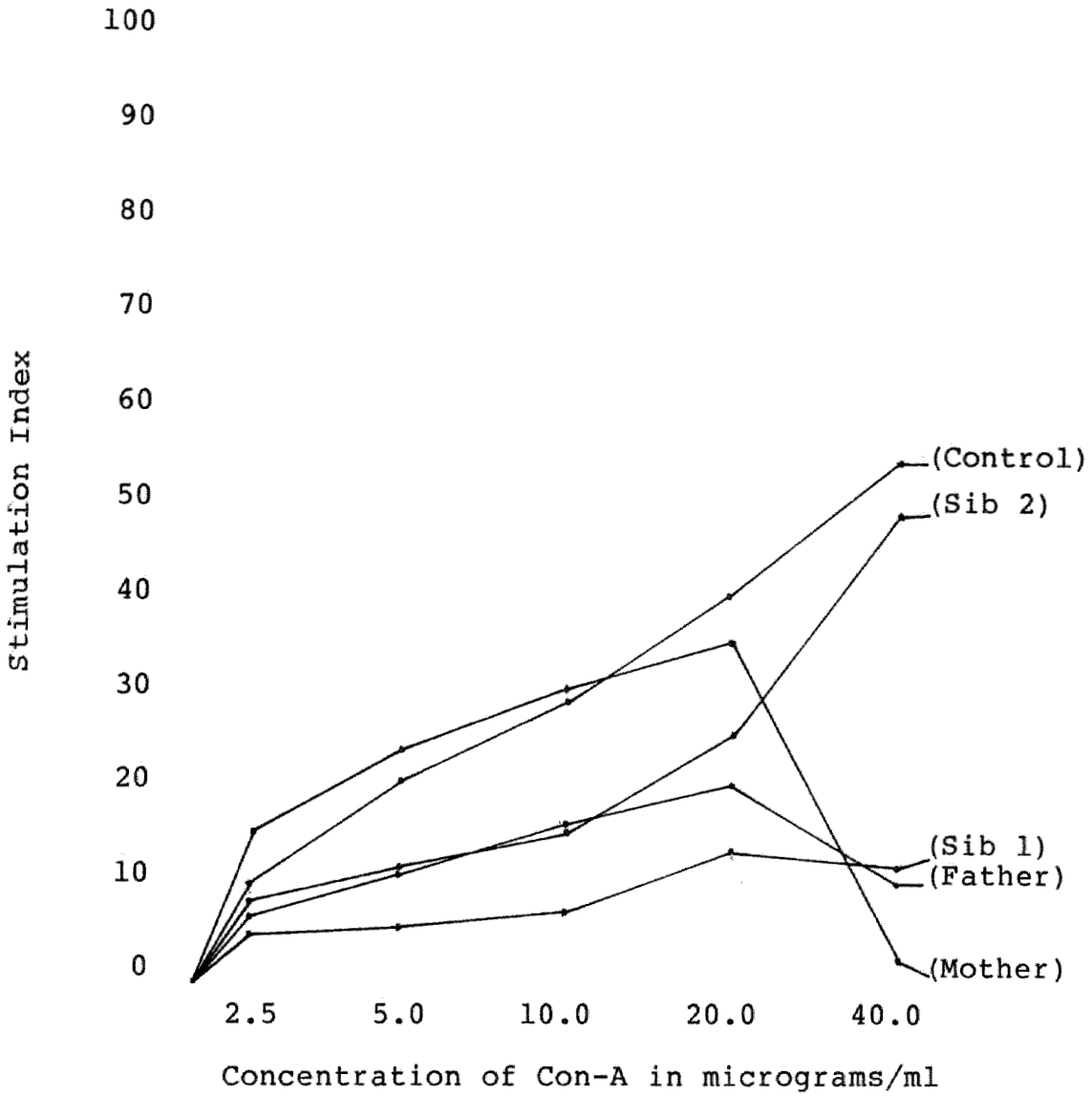

Figure 2. Stimulation indexes for the mitogen Con-A.

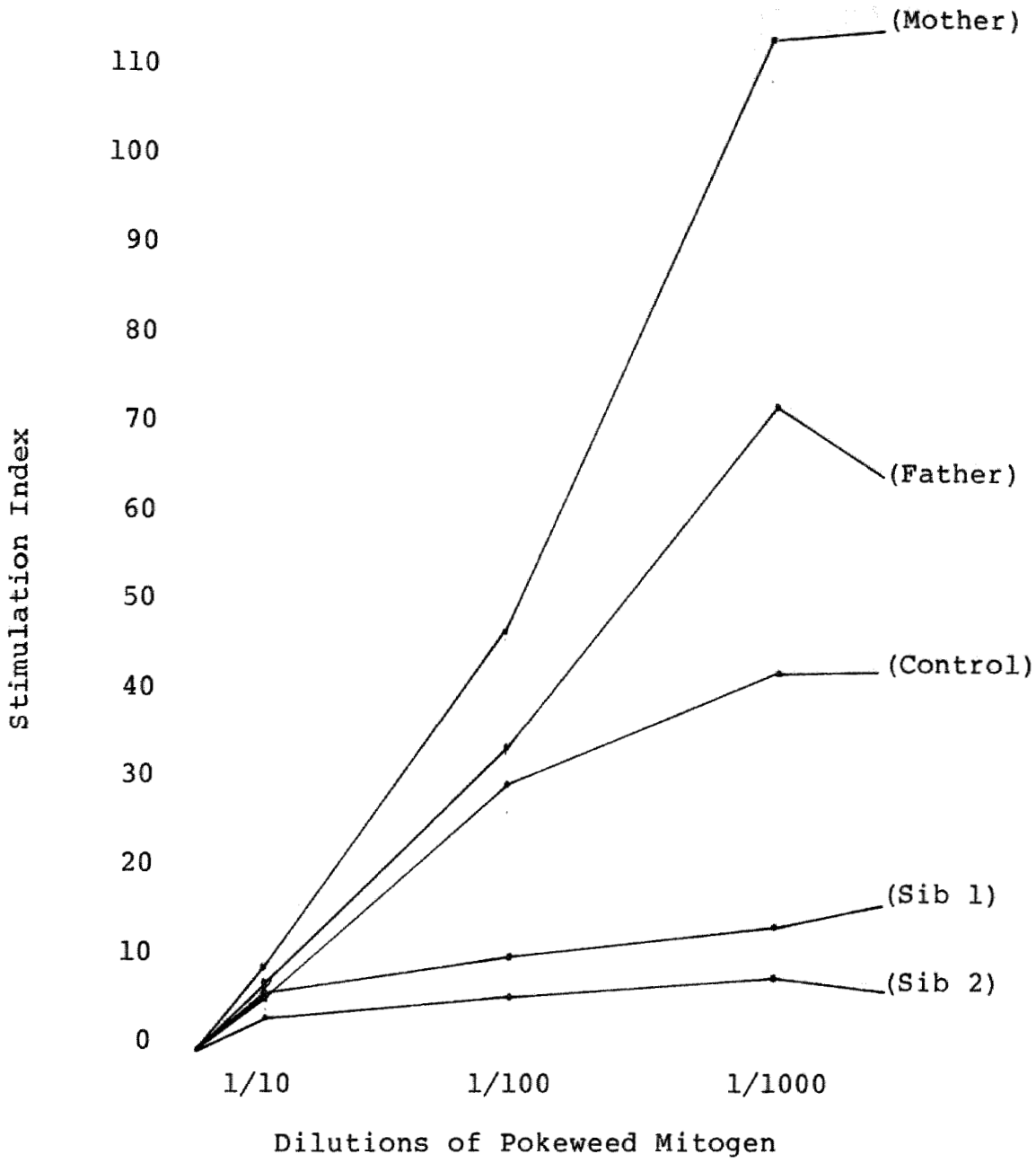

Figure 3. Stimulation indexes for Pokeweed Mitogen (PWM).

Table 5. Counts per minute of harvested cells in (MLC). The (x) rows indicate stimulator cells, while the horizontal rows represent responder cells. Underlined numbers are the base line counts used in figuring stimulation indexes for Table 6.

	Father(x)	Mother(x)	Sib 1(x)	Sib 2(x)	Control(x)	PHS(x)
Father	397	1042	1086	1613	629	263
Mother	1269	331	369	867	2974	230
Sib 1	227	242	165	376	1186	880
Sib 2	287	220	207	346	607	1751
Control	691	188	659	498	227	230
PHS	307	255	277	270	230	---

Table 6. Stimulation indexes in the MLC.

	Father(x)	Mother(x)	Sib 1(x)	Sib 2(x)	Control(x)
Father	---	3	3	4	2
Mother	4	---	1	3	9
Sib 1	1	1	---	2	7
Sib 2	0	0	0	---	2
Control	3	0	3	2	---

siblings. The stimulating capacity of the BS cells (vertical rows) do not differ significantly from that of the control lymphocytes.

Due to the small number of tests performed it was impossible to statistically analyze the results of this study. Ideally more controls would have been included but due to the complicated nature of most of the tests and the availability of only one technician the study had to be designed as it was. As a case study these observations will hopefully prove to be of value.

DISCUSSION

Patients' Medical History

The medical history of these two BS siblings is complex. Sibling 1 was born July 14, 1977, with a birth weight of six pounds. He was diagnosed at age one as having BS. There were gastrointestinal complications. Failure to thrive was apparent at an early age. At 16 months growth hormones were normal but x-rays estimated a retarded bone age to be similar to that of a newborn. A quantitative immunoglobulin assay revealed that this sibling possessed abnormally low concentrations of both IgG and IgM antibody. The thyroid stimulating hormone (TSH) was low. Glucose, total protein and carotene concentrations were all low. Electrolyte levels were normal. A 24-hour urine amino acid analysis showed this patient to possess abnormally low

levels of nearly every amino acid assayed. The significance of this is not known.

At 18 months a chromosome analysis at Mayo Clinic reported no apparent abnormalities. Sibling 1's medical history has a plethora of such observations as upper respiratory infection, otitis media and failure to thrive. Due to recurrent otitis media a myringotomy with tubes was performed.

This sibling seems to be especially sun sensitive. Bouts with ezcematous, erythematous, impetiginous rashes were treated with hydrocortisone. At age six he was admitted to the hospital with entire body scaling (including the scalp), an oozing from flexural skin and fissures and crusting in those areas.

Chromosome work was repeated at this time at the University of Iowa. There was no change in this sibling or in sibling 2 who had also been diagnosed as BS. No increased chromosomal breakage or increased sister chromatid exchange (SCE) were noted.

Sibling 2 was born July 22, 1978, weighing five pounds four ounces and having apgars of 7 and 7. He had a history of vomiting and diarrhea with attendant hypokalemia. Again diagnosed as failure to thrive he was transferred to Mayo Clinic. A 24-hour urine amino acid assay revealed that this patient (like his sibling) to have low concentrations of most amino acids assayed.

Immunoglobulin levels revealed him to have low concentrations of IgG and IgA.

At one year the characteristic butterfly facial rash was noted and a genetics consult for BS was suggested. At 18 1/2 months sibling 2 was diagnosed as BS in spite of the fact that there was no increase in chromosomal breakage. He had a continuing history of gastrointestinal, respiratory and ear problems.

A letter from the Iowa Department of Health dated September 1979 states that these siblings have many features which fit the diagnosis of BS: Pre- and post-natal growth failure, mild microencephaly (no dolichocephaly noted), malar rash with livedo reticularis. They noted sibling 1's extreme light sensitivity and stated that there was no malformation of the hands or feet. It was also observed that there was no Jewish ancestry--the mother being Dutch-Irish and the father English-Irish-German. The disease is most common in children of Jewish ancestry and seems to have been previously limited to those of Ashkenozim heritage (German et al. 1977).

A physical exam at the University of Iowa in April of 1983 described both boys as short for their ages with erythematous rash on their faces. Both were stated as having high palates and knock knees. Both were below the third percentile in height and weight. Neither sibling exhibited cafe au lait spots which is a common feature in

many BS individuals. No hypogonadism was noted.

On August 6, 1984, the parents received a letter from the Division of Medical Genetics at the University of Iowa. The letter stated that the geneticists did not believe these siblings have BS since they hadn't been able to demonstrate SCE's of increased frequency. They also felt that these children were not at increased risk for malignancy.

According to the tests performed in this study the parents' and sibling's neutrophils appear to be operating normally and their chemotactic and phagocytic abilities seem to be within acceptable limits. The T- and B-cell populations also fall in normal ranges along with T-cell subsets. An abnormal response was observed with the mitogen stimulation of lymphocytes. Sibling 1 showed a somewhat impaired response to both PHA and Con-A while sibling 2 had a better response to both mitogens. According to both Weemaes et al. (1979) and Hutteroth et al. (1975), BS individuals show a lower response to PHA than normal controls, though in Weemaes' study of two families one family did have normal response to PHA which leads one to think that this trait may be variable.

Pokeweed mitogen response was poor in both siblings. Previous studies (Weemaes et al. 1979; Hutteroth et al. 1975) indicate this response would be expected. The reason for lack of response may lie with the T4 (helper) and

T8 (suppressor) T-cell subsets. Suppressor cells bearing receptors for IgG may suppress B-cell differentiation promoted by PWM (Schoen 1967). Helper cells bear IgM receptors that "preactivate" B-cells so that PWM can transform them into plasma cells. In the presence of PWM the suppressor cells release soluble factors that suppress B-cell proliferation. This suggests that suppressor cells can suppress induction of polyclonal B-cell differentiation by acting indirectly on helper cells.

Studies by Taniguchi et al. (1982) suggest that peripheral blood lymphocytes in BS patients are defective in both B-cell ability to produce immunoglobulins and in T-cell help for B-cell differentiation, as if functional maturation of the lymphocytes were arrested at an early stage of immune ontology.

Due to the lack of B-cell stimulation by PWM one might correctly assume that BS subjects will be deficient in one or more classes of immunoglobulins. This fact is born out by both of the siblings in this study. Such a deficiency in BS may prove important in helping to explain the clinical picture and pathogenesis of this disease. The low gamma globulin levels may even be a factor in the development of leukemia. Children with agammaglobulinemia are known to develop acute lymphatic leukemia (Page et al. 1963).

While hypogonadism is not found in these two siblings

it is a clinical finding in many BS subjects. Shabtai and Halbrecht (1980) describe a BS patient in which there was increased chromosomal breakage and SCE. They found this man to be infertile at age 33 due to hypogonadism. Fifteen percent of this patient's cells were missing the Y chromosome. According to Padre-Mendoza et al. (1979) a missing Y has been implicated in the development of hematologic malignancies, especially in young people.

Results of the mixed lymphocyte culture in this study concur with results one would expect to find in a BS patient. The siblings were obviously poor responders but average stimulators. The ability of lymphocytes from persons with BS to react with allogenic cells in this test was also observed in previous works (Weemaes et al. 1979; Hutteroth et al. 1975). Past studies demonstrated, as did ours, that BS lymphocytes responded less vigorously to normal stimulator lymphocytes than did normal responder lymphocytes to normal stimulator lymphocytes. There seems to be both decreased T-cell function (decreased responder activity in MLC) and B-cell abnormalities (shown in decreased serum concentration of at least one immunoglobulin class) in BS.

Hutteroth et al. (1975) suggest that immunological abnormalities in BS may be secondary to a disturbance in cellular proliferation. They believe that this disturbance could cause a specific pattern of immune defects the

severity of which would depend on the nature of the defect and the class of cells affected. Miller and Todaro (1969) speculate that a defective immune response in BS may play an important role in the incidence of malignancy.

There is no direct evidence as yet correlating SCE and immune disfunction, but increased SCE is a cardinal feature in the BS patient. If the SCE's involve any of the immunoglobulin genes, then obviously immune function would be effected. This type of exchange has not been documented.

Santesson et al. (1969) stated that SCE levels in normal B-cells were significantly lower than those in the T-cells of the same individuals. Others observed (Lindblad and Lambert 1981; German et al. 1977) that a population of PHA stimulated lymphocytes exhibited a normal level of SCE in some BS individuals. These results were thought to be indicative of a dimorphism which could be ascribed to the low SCE values that they found in BS B-cells as compared to normal B-cells.

Block et al. (1982) experimented with SCE levels in T- and B-cells from five BS patients. They stimulated B-cells with mitogens specific for them such as liposaccharide and Epstein-Barr virus. Their results demonstrated SCE levels in B-cells of BS patients to be very high and similar to those of T-cells. They concluded that there was no dimorphism in regard to SCE in the lymphocyte population of the BS cases studied.

Schonberg and German (1980) suggest that elevated SCE's in persons with BS are a molecular defect related to DNA metabolism. While other studies (Selsky et al. 1979; Thompson and Holliday 1983) have shown that the DNA repair mechanisms are intact in the BS patient, Schoenberg and German state that the various chromosome abnormalities in BS may result from an excessive accumulation of lesions in the DNA on which a repair system must act thereby overloading the system. They felt that such lesions might result from the action of a genetically defective enzyme leading to the production of a molecule with mutagenic properties.

Barnabei and Kelly (1982) suggest that BS fibroblasts synthesize a metabolite capable of increasing SCE frequency in normal fibroblasts. The nature of such a factor remains unknown. Also of interest is the fact that when BS cells are grown in medium preconditioned by normal cells a reduction of SCE's is noted. Schmidt-Preu et al. (1981) speculate that this "corrective factor" from normal cells may be a gene product which is defective or absent in BS cells.

Warren et al. (1981) thought it conceivable that when the bl gene is homozygous it may have mutator activity. Their experiment demonstrated that fibroblasts from BS had a 5- to 10-fold increase in spontaneous mutations over control fibroblasts. They concluded that when the gene bl is homozygous it may have significant mutator activity

associated with it. They surmised that because the growth of an individual is determined by cell numbers, that cell loss due to inviable somatic mutations added to the slow mitotic rate found in BS may play an important role.

According to Thompson and Holliday (1983) there seems to be a possible relationship between DNA repair and cellular aging. The life span of fibroblasts from BS subjects are half that of normal fibroblasts. Enzymes are constantly recognizing and repairing lesions in DNA. If errors are accumulating exponentially, maintenance mechanisms may break down and cell death will follow. Evidence shows that various deleterious genetic changes accumulate near the end of a cell's life including chromosome abnormalities, mutations and loss of fidelity of DNA polymerases. The theories of Warren et al., Thompson and Holliday as stated above fit very nicely with the genetic picture manifested by the BS patient.

Giannelli et al. (1981) also felt that BS defect may express itself during DNA synthesis. They noted that the levels of unscheduled DNA synthesis (UDS) upon exposure to ultraviolet light may be related to anomalies in excision repair. It is suggested by them that BS cells contain an intracellular diffusible factor which influences the initial rate of UDS and that this gene product may compete with the normal allele and modify the initial rate of UDS induced by UV rays.

CONCLUSIONS

There appear to be many theories as to the etiology of BS. At present most of the observations are speculation based on clinical and laboratory data. The clinical picture of the siblings in this study is noted in Table 7 and is compared to other known cases of BS.

The results of this immunological study seem to correlate with those of previous studies. As expected from past work with BS our patients had normal T- and B-cell numbers and T-cell subsets. Though there was no literature concerning chemotaxis and NBT findings both siblings and parents demonstrated normal abilities. While the mitogen reactions were varied, the results of the MLC and pokeweed stimulation gave classic BS responses.

Bloom's Syndrome is difficult to diagnose. Intrauterine and postnatal growth retardation is a consistent feature. Skin lesions may vary considerably. Such minor abnormalities such as body asymmetry and clino (micro) dactyly might suggest a diagnosis of Silver-Russell Syndrome in some patients. BS must also be differentiated from other types of dwarfism and skin abnormalities like Rothmund-Thompson Syndrome and Cockayne's Syndrome (Vanderschueren-Lodewycky et al. 1984).

Decreased immunoglobulin levels are characteristic of BS, but may vary as seen in our two siblings. While no direct relationship has as yet been established between

Table 7. Symptoms found in diagnosed BS individuals as compared to the symptoms noted in the two siblings of this study.

Symptoms of BS:	Present	Absent
Growth retardation (pre- & post-natal)	x	
Sun sensitivity	x	
Erythematous rash	x	
Increased SCE		x
Abnormal immunoglobulin levels	x	
Microcephaly	x (mild)	
Doliochocephaly		x
Cafe au lait spots		x
Hypogonadism		x
Impaired response PWM	x	
Poor responder MLC	x	
Increased infections	x	

immunoglobulin deficiency and the chronic infections which seem to plague the BS patient, it can't help but be a factor as evidenced by repeated infections in our study's siblings.

The very high rate of SCE's is now considered an essential feature of BS. This makes it difficult to give a definite diagnosis of BS in the subjects of this study. While they have many of the classic symptoms of BS one critical factor is absent, an increased number of SCE's.

The results of this study do agree, for the most part, with those found in other BS studies' but similar results may also be found in other genetically defective syndromes. There are definite abnormalities in the immune systems of these siblings and they do fit the basic clinical description of the typical BS patient. Do these boys have BS? The answer is still equivocal.

In future years possibly a chromosome instability will manifest itself but without the demonstration of increased SCE's one must agree that, at least genetically, these siblings' risk of cancer is not increased at this time. The chronic infections these patients face are not life threatening and have generally been controlled with proper treatment.

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